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HUMAN GENOME EPIDEMIOLOGY (HuGE) REVIEW

Systematic Review and Meta-Analysis of the Association between β_2-Adrenoceptor Polymorphisms and Asthma: A HuGE Review

Ammarin Thakkinstian[1], Mark McEvoy[2], Cosetta Minelli[3], Peter Gibson[4], Bob Hancox[5], David Duffy[6], John Thompson[3], Ian Hall[7], Joel Kaufman[8], Ting-fan Leung[9], Peter Joseph Helms[10], Hakon Hakonarson[11], Eva Halpi[11], Ruth Navon[12], and John Attia[2]

[1] Clinical Epidemiology Unit, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.
[2] Centre for Clinical Epidemiology and Biostatistics, University of Newcastle, Newcastle, New South Wales, Australia.
[3] Centre for Biostatistics and Genetic Epidemiology, Department of Health Sciences, University of Leicester, Leicester, United Kingdom.
[4] Department of Respiratory and Sleep Medicine, Hunter Medical Research Institute, John Hunter Hospital, Newcastle, New South Wales, Australia.
[5] Department of Respiratory Medicine, Waikato Hospital, Hamilton, New Zealand.
[6] Queensland Institute of Medical Research, Brisbane, Queensland, Australia.
[7] Division of Therapeutics and Molecular Medicine, University Hospital, Nottingham, United Kingdom.
[8] Department of Environmental and Occupational Health Sciences, School of Public Health and Community Medicine, University of Washington, Seattle, WA.
[9] Department of Paediatrics, The Chinese University of Hong Kong, Hong Kong, Special Administrative Region, China.
[10] Department of Child Health, Medical School, University of Aberdeen, Aberdeen, United Kingdom.
[11] deCODE Genetics, Inc., Reykjavik, Iceland.
[12] Department of Genetics, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

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A number of studies have investigated two common polymorphisms in the β_2-adrenoceptor gene, *Arg/Gly16* and *Gln/Glu27*, in relation to asthma susceptibility. The authors performed a meta-analysis of each polymorphism, as well as haplotype analysis, for adult and pediatric populations separately, using published data, supplemented by additional data requested from the original authors. Individual analysis detected no effect of *Arg/Gly16* in adults but did suggest a recessive protective effect of *Gly16* for children, with an odds ratio of 0.71 (95% confidence interval (CI): 0.53, 0.96) compared with the other genotypes. Results for *Gln/Glu27* in adults seem to indicate that heterozygotes are at decreased risk of asthma than either homozygote (odds ratio = 0.73, 95% CI: 0.62, 0.87), although the studies are heterogeneous; in children, the *Glu/Glu* genotype has a decreased risk of asthma (odds ratio = 0.60, 95% CI: 0.35, 0.99) compared with the other genotypes. Despite the proximity of these two polymorphic sites, the linkage disequilibrium coefficient of 0.41 was not high ($p < 0.001$). Haplotype analysis suggests that there may be an interaction between the two sites, with a lower risk of asthma associated with the *Glu27* allele (compared with *Gln27*), and that this risk is modified by the allele at position 16.

asthma; epidemiology; genetics; haplotypes; linkage disequilibrium; meta-analysis; polymorphism, genetic; receptors, adrenergic

Abbreviations: CI, confidence interval; LR, likelihood ratio; OR, odds ratio; SNP, single-nucleotide polymorphism.

Editor's note: *This paper is also available on the website of the Human Genome Epidemiology Network (http://www.cdc.gov/genomics/hugenet/).*

One of the main thrusts of genetic epidemiology is to understand the genetic contribution to complex diseases such as cardiac disease, diabetes, and asthma. One of the

Correspondence to Dr. John Attia, Centre for Clinical Epidemiology and Biostatistics, University of Newcastle, Newcastle, NSW 2300, Australia (e-mail: John.Attia@newcastle.edu.au).

most popular study designs in this area is a molecular association study in which a polymorphism is linked to the disease outcome, either in cases and controls or in a cohort. These studies are often limited by small sample sizes (1), so there is a role for meta-analysis in pooling these studies, particularly to detect the small effect sizes that may be associated with these polymorphisms.

The β_2-adrenoceptor gene is a key gene to study in asthma. β_2-Adrenoceptors are present on many airway cells, including smooth muscle cells which are hyperreactive in asthma, and β_2-adrenoceptor agonists form a major treatment class in asthma. Functional polymorphisms of this gene may influence both disease susceptibility and treatment response in asthma.

A number of studies have investigated polymorphisms in the β_2-adrenoceptor gene in relation to asthma. Two common polymorphisms are *Arg/Gly16* and *Gln/Glu27*; in the former polymorphism, glycine is substituted for arginine at codon 16 (Arg16 → Gly) and, in the latter, glutamic acid is substituted for glutamine at codon 27 (Gln27 → Glu) (2, 3). In vitro studies indicate that the *Gly16* allele enhances agonist-induced down regulation of the receptor, whereas the *Glu27* allele enhances resistance to down regulation (4, 5). It is plausible that these differences in receptor regulation influence the reactivity of airway smooth muscle in response to airway inflammation and thereby alter the risk of asthma. However, epidemiologic studies have yielded conflicting results, with the direction of the effects not always congruent with the in vitro results. Several narrative reviews of these two polymorphisms and asthma (4–6) have been conducted; however, neither a magnitude nor a mode of gene effect was provided in these reviews. Furthermore, new studies that examine this association have been reported since those reviews, and there have been new developments in the methodology of meta-analysis of genetic studies (1, 7, 8). We therefore performed a systematic review of the association between *Arg/Gly16* and *Gln/Glu27* and asthma with the following objectives: first, to estimate allele frequencies; second, to ascertain if there is an effect of these polymorphisms on asthma susceptibility, and if so to estimate the magnitude of that effect and the possible mode of inheritance (1, 7, 8); third, to determine linkage disequilibrium between these two polymorphisms; and fourth, to infer haplotypes of these polymorphisms and link them with asthma susceptibility.

MATERIALS AND METHODS

Search strategy

Embase and Medline databases (from January 1966 to March 2004) were searched using the Embase, PubMed, and Ovid search engines. The search strategy for allele frequency was as follows: beta2* AND prevalence AND gene. The search strategy for association between gene polymorphisms and asthma was the following: asthma AND (beta receptor or beta-2 or adrenoceptor) AND (polymorph* or mutation* or variant* or genotype*). Searching was performed in duplicate by two independent reviewers (A. T. and M. M.).

Inclusion criteria

For allele frequency, any human studies that estimated the prevalence of β_2-adrenoceptor polymorphisms at codon 16 (*Arg/Gly16*) and/or codon 27 (*Gln/Glu27*) and reported on ethnically homogeneous populations were included, regardless of size. For assessing association, human studies, regardless of sample size, were included if they met the following criteria:

- β_2-Adrenoceptor polymorphisms at codon 16 (*Arg/Gly16*) and/or codon 27 (*Gln/Glu27*) were determined. The wild-type alleles for these two polymorphisms were *Arg* and *Gln*, respectively.
- The outcome was asthma (incident or prevalent), and there were at least two comparison groups, for example, asthma versus control (nonasthma) groups.
- Participants could be either children or adults, but results should be reported separately.
- There were sufficient results for extraction of data, that is, number of subjects for each genotype in asthma and control groups. Where eligible papers had insufficient information, we contacted authors by e-mail for additional information.

The reference lists of the articles retrieved were also reviewed to identify publications on the same topic. The most complete and recent results were used where there were multiple publications from the same study group.

Data extraction

Data were extracted independently and in duplicate by two reviewers (A. T. and M. M.) who used a standardized data extraction form. Any disagreement was adjudicated by a third author (J. A.). Covariables, such as mean age, gender, and ethnicity, were also extracted for each study.

Quality score assessment

The quality of studies was also independently assessed by the same two reviewers who used quality assessment scores that were modified from our previous meta-analysis of molecular association studies (7) (appendix table 1). These scores were based on both traditional epidemiologic considerations and genetic issues (1). Total scores ranged from 0 (worst) to 13 (best).

Statistical analysis

Data analyses were performed as follows. First, the frequency of *Arg16* and *Gln27* alleles in various ethnic groups was estimated by the inverse variance method, as described in the Appendix.

Second, estimation of the gene effect on asthma was performed by a logistic regression approach described previously (8). In brief, the steps were as follows. Hardy-Weinberg equilibrium was assessed for each study by use of the χ^2 test or Fisher's exact test, where appropriate, and only in control groups. A Q test for heterogeneity was performed separately for three odds ratios (ORs), that is, *Gly/Gly* versus

Arg/Arg (OR_1), *Arg/Gly* versus *Arg/Arg* (OR_2), and *Gly/Gly* versus *Arg/Gly* (OR_3) for the *Arg/Gly16* polymorphism and *Glu/Glu* versus *Gln/Gln* (OR_1), *Gln/Glu* versus *Gln/Gln* (OR_2), and *Glu/Glu* versus *Gln/Glu* (OR_3) for the *Gln/Glu27* polymorphism. If there was heterogeneity on at least one of these odds ratios, the cause of heterogeneity was explored by fitting a covariable (e.g., ethnicity, age, gender, or quality score) in a meta-regression model (9–11). If there was no heterogeneity, logistic regression analysis with the fixed-effect model was used to determine the gene effect; otherwise, the random-effect model was used to pool. A likelihood ratio test was used to gauge whether the overall gene effect was significant. If the main effect of the genotype was statistically significant, further comparisons of OR_1, OR_2, and OR_3 were explored. These pairwise differences were used to indicate the most appropriate genetic model as follows.

1. If $OR_1 = OR_3 \neq 1$ and $OR_2 = 1$, then a recessive model is suggested.
2. If $OR_1 = OR_2 \neq 1$ and $OR_3 = 1$, then a dominant model is suggested.
3. If $OR_2 = 1/OR_3 \neq 1$ and $OR_1 = 1$, then a complete overdominant model is suggested (also referred to as a ''homozygous model'' or ''heterosis'').
4. If $OR_1 > OR_2 > 1$ and $OR_1 > OR_3 > 1$ (or $OR_1 < OR_2 < 1$ and $OR_1 < OR_3 < 1$), then a codominant model is suggested.

Third, the gene effect was estimated by use of a newer, ''parsimonious'' approach detailed elsewhere (C. Minelli et al., University of Leicester, unpublished manuscript). In brief, this approach summarizes the genetic model in terms of a parameter lambda (λ), which is the ratio between $\log(OR_1)$ (*Glu/Glu* vs. *Gln/Gln*) and $\log(OR_2)$ (*Gln/Glu* vs. *Gln/Gln*). This parameter, which represents the heterozygote effect as a proportion of the homozygote variant effect, captures information about the genetic mode of action as follows: a recessive model if $\lambda = 0$, a dominant model if $\lambda = 1$, a codominant model if $\lambda = 0.5$, and homozygous or overdominant if λ is greater than 1 or less than 0. The two log odds ratios are modeled as either fixed or random effects, as described in the second statistical analysis enumerated above.

Once the best genetic model is identified, this model is used to collapse the three genotypes into two groups (except in the case of a codominant model) and to pool the results again. Sensitivity analyses were performed by including or excluding studies not in Hardy-Weinberg equilibrium.

Fourth, with haplotype analysis, the haplotype frequencies of *Arg/Gly16* and *Gln/Glu27* polymorphisms were inferred using the expectation-maximization algorithm (12). The odds ratio was then estimated by use of the profile likelihood. The linkage disequilibrium coefficient was then estimated (13). The likelihood ratio test was used to test whether the linkage disequilibrium was significant.

All analyses were performed using Stata software, version 8.0 (14), apart from the parsimonious approach, for which WinBugs 1.4 (15) with vague prior distributions was used. A p value of less than 0.05 was considered statistically significant, except for tests of heterogeneity where a level of 0.10 was used.

RESULTS

For pooling allele frequency, 67 studies were identified, of which 16 (16–31) reported separate information for defined ethnic groups. Fourteen studies (2, 3, 32–43) retrieved from the search for gene effect were also included.

Allele frequencies

Arg allele. To estimate the pooled frequency, we used data only from control groups where a case-control design was used or from the entire group where a cohort design was used. Twenty-six studies (2, 3, 17, 18, 20–22, 24, 26–43) reported *Arg* allele frequencies (table 1), with 13 studies of Caucasian adults, three of Caucasian children, four of Black adults, six of Oriental adults, two of Oriental children, and one of Semite (Jews/Arabs) adults. Of these, six were not in Hardy-Weinberg equilibrium, leaving 12 studies of Caucasians, three of Blacks, and seven of Orientals for pooling.

There was heterogeneity among the 12 Caucasian studies ($\chi^2_{11} = 109.96$, $p < 0.001$). The pooled frequency using the random effects model was 42.0 percent (95 percent confidence interval (CI): 38.4, 45.7). The pooled frequency among Blacks was 49.2 percent (95 percent CI: 45.7, 52.7), and this estimate was homogeneous ($\chi^2_2 = 0.24$, $p = 0.89$). There was heterogeneity among Oriental studies ($\chi^2_6 = 18.79$, $p = 0.01$), and the pooled frequency was 56.2 percent (95 percent CI: 51.9, 60.6).

Gln allele. Twenty-six studies (3, 16, 17, 19, 20, 22–25, 27–43) reported the frequency of the *Gln/Glu27* polymorphism, 12 studies of Caucasian adults, three of Caucasian children, three of Black adults, seven of Oriental adults, two of Oriental children, one of Jewish adults, and one of Polynesian adults (table 2). Three studies, all of Caucasians, did not observe Hardy-Weinberg equilibrium and were not included in pooling.

There was heterogeneity among the 10 Caucasian studies ($\chi^2_9 = 437.77$, $p < 0.001$), and the pooled frequency was 59.6 percent (95 percent CI: 53.6, 65.6). All Black studies were homogeneous ($\chi^2_2 = 1.08$, $p = 0.58$), and the pooled frequency with the fixed model was 81.3 percent (95 percent CI: 79.7, 83.0). Seven Oriental studies were also homogeneous ($\chi^2_6 = 7.26$, $p = 0.30$), and the pooled frequency was 91.9 percent (95 percent CI: 90.9, 92.9).

Assessing association between gene polymorphisms and asthma

Across both Embase and Medline databases, 435 studies were identified in total, of which 113 were duplicates, leaving 322 study abstracts that were reviewed. From these, 30 studies seemed to be relevant, and therefore the full papers were retrieved. Sixteen studies were judged to have met the inclusion criteria, of which eight provided complete data in the paper. Requests for additional data on the other eight studies were made, of which four were granted. Two additional studies (36, 43) were identified by a known expert (D. D.), and the authors provided additional data. The characteristics of the adult and pediatric study populations, for

TABLE 1. Estimation of the pooled prevalence of the *Arg* allele

Subjects, first author (reference no.)	Hardy-Weinberg equilibrium (*p* value)	Total no.	*Arg* allele frequency (no.)	% with *Arg* allele
Caucasian adults*				
Santillan (3)	0.07	1,208	520	43
Barr (2)	0.50	274	164	60
Holloway (32)	0.30	182	73	40
Dewar (33)	0.32	1,268	489	39
Arnaiz (34)	0.02†	102	42	41
Reihsaus (35)	0.04†	112	30	27
Hakonarson (36)	0.75	362	127	35
Rosmond (17)	<0.001†	534	238	45
Dallongeville (20)	0.53	2,258	857	38
Tang (21)	0.06	248	95	38
Aynacioglu (22)	0.84	208	84	40
Weir (30)	1.00	168	102	61
Xie (31)	<0.05†	376	172	46
Caucasian children*				
Martinez (37)	0.90	538	206	38
Binaei (38)	0.13	310	135	44
Hopes (39)	0.12	838	279	33
Black adults‡				
Kotanko (18)	<0.05†	162	63	33
Tang (21)	0.51	286	144	50
Candy (24)	0.37	246	119	48
Xie (31)	1.00	246	120	49
Oriental adults§				
Wang (42)	0.50	272	140	51
Sugaya (26)	<0.05†	414	165	40
Chang (27)	0.11	260	137	53
Kim (28)	0.37	178	115	65
Iwamoto (29)	0.71	238	115	48
Xie (31)	0.69	208	122	59
Oriental children§				
Leung (41)	0.48	140	81	58
Lin (40)	0.06	298	182	61
Jewish/Arab adults				
Shachor (43)	0.45	222	101	45.5

* Pooled prevalence (%): 42 (95% confidence interval (CI): 38.4, 45.7).
† Not included in pooled prevalence.
‡ Pooled prevalence: 49.2 (95% CI: 45.7, 52.7).
§ Pooled prevalence: 56.2 (95% CI: 51.9, 60.6).

TABLE 2. Estimation of the pooled prevalence of the *Gln* allele

Subjects, first author (reference no.)	Hardy-Weinberg equilibrium (*p* value)	Total no.	*Gln* allele frequency (no.)	% with *Gln* allele
Caucasian adults*				
Arnaiz (34)	<0.05†	102	54	53
Santillan (3)	0.12	1,208	972	81
Holloway (32)	0.13	182	107	59
Dewar (33)	0.58	1,260	656	52
Reihsaus (35)	0.19	112	57	51
Hakonarson (36)	0.09	398	208	52
Rosmond (17)	0.45	532	314	45
Heckbert (19)	0.81	8,882	5,069	57
Dallongeville (20)	<0.001†	2,982	1,321	44
Aynacioglu (22)	0.81	208	142	68
Weir (30)	1.00	168	90	54
Xie (31)	0.06	376	245	65
Caucasian children*				
Martinez (37)	0.69	538	343	64
Hopes (39)	0.38	838	433	52
Binaei (38)	<0.05†	310	250	81
Black adults‡				
Heckbert (19)	0.64	1,616	1,315	81
Candy (24)	0.75	246	204	83
Xie (31)	0.78	246	195	79
Oriental adults§				
Wang (42)	1.00	272	248	91
Kawamura (16)	0.16	838	772	92
Kahara (23)	1.00	248	233	94
Chang (27)	0.55	260	240	92
Kim (28)	0.59	176	156	89
Iwamoto (29)	1.00	238	221	93
Xie (31)	1.00	208	193	93
Oriental children§				
Leung (41)	1.00	140	125	89
Lin (40)	1.00	298	267	90
Jewish/Arab adults				
Shachor (43)	0.66	218	150	69
Polynesian				
Duarte (25)	0.51	2,044	1,944	95

* Pooled prevalence (%): 59.6 (95% confidence interval (CI): 53.6, 65.6).
† Not included in pooled prevalence.
‡ Pooled prevalence: 81.3 (95% CI: 79.7, 83.0).
§ Pooled prevalence: 91.9 (95% CI: 90.9, 92.9).

example, mean age, gender, ethnicity, type of subjects, and allele frequency, are given in table 3.

Arg/Gly16 polymorphism. Adult asthma. Nine studies (2, 3, 32–36, 42, 43) determined the association between *Arg/Gly16* and asthma in adults (table 4). Total sample sizes for asthma and control groups were 1,331 and 1,872, respectively. Within the asthma group, the mean age was 41 (standard deviation: 11) years, and 49 percent were females. Within the control group, the mean age was 39 (standard deviation: 11) years, and 35 percent were females.

TABLE 3. General characteristics of studies included in pooling gene effects

Subjects, first author (reference no.)	Year	Study design	Race	Mean age (years)	% female	Quality score
Adults						
Shachor (43)	2003	Case-control	Jewish/Arab	38	53.0	5
Arnaiz (34)	2003	Cohort	Caucasian	28	1.9	9
Santillan (3)	2003	Case-control	Caucasian	37.3	15.0	13
Barr (2)	2001	Case-control	Caucasian	58.4	64.9	10
Wang (42)	2001	Case-control	Asian	33.0	61.7	13
Hakonarson (36)	2001	Case-control	Caucasian	47.5	56.3	6
Holloway (32)	2000	Case-control	Caucasian	31.4	54.9	6
Dewar (33)	1998	Cross-sectional	Caucasian	18–70*	54.0	6
Reihsaus (35)	1993	Case-control	Unknown	46		5
Children						
Martinez (37)	1997	Cross-sectional	Caucasian	10.8		9
Hopes (39)	1998	Cross-sectional	Caucasian	10.5		5
Leung (41)	2002	Case-control	Asian	10.8	55.0	5
Binaei (38)	2003	Case-control	Caucasian			1
Lin (40)	2003	Cross-sectional	Asian	13.9		9

* Range.

The seven studies (2, 3, 32, 33, 36, 42, 43) that observed Hardy-Weinberg equilibrium were pooled. Heterogeneity was checked for OR_1 (*Gly/Gly* vs. *Arg/Arg*), OR_2 (*Arg/Gly* vs. *Arg/Arg*), and OR_3 (*Gly/Gly* vs. *Arg/Gly*). Results indicated heterogeneity for OR_1 and OR_2 but not for OR_3 (for OR_1: $\chi^2_6 = 14.14$, $p = 0.03$; for OR_2: $\chi^2_6 = 13.98$, $p = 0.03$; for OR_3: $\chi^2_6 = 10.38$, $p = 0.11$). Race was explored as a potential cause; however, heterogeneity was still present in all odds ratios after excluding the one study of Asians (42) and the one study of Semites (for OR_1: $\chi^2_4 = 8.85$, $p = 0.07$; for OR_2: $\chi^2_4 = 9.76$, $p = 0.04$; for OR_3: $\chi^2_4 = 7.84$, $p = 0.10$). Hence, these seven studies were pooled by use of logistic regression with the random-effects model. The overall gene effect was not significant (likelihood ratio (LR) = 0.01, $p = 0.99$), with the estimated OR_1, OR_2, and OR_3 being 1.00 (95 percent CI: 0.80, 1.24), 0.99 (95 percent CI: 0.81, 1.22), and 1.01 (95 percent CI: 0.85, 1.20), respectively (table 5). Analysis using the parsimonious approach yielded very similar results: $OR_1 = 1.01$ (95 percent CI: 0.79, 1.32), $OR_2 = 1.00$ (95 percent CI: 0.79, 1.30), and $\lambda = 0.15$ (95 percent CI: −4.15, 4.99).

Sensitivity analysis was performed by including the two studies (34, 35) that did not observe Hardy-Weinberg equilibrium; the results were similar in showing no genetic effect ($LR_2 = 0.41$, $p = 0.96$).

Childhood asthma. Five studies (37–41) determined the association between the *Arg/Gly16* polymorphism and asthma in children (table 4), and all observed Hardy-Weinberg equilibrium. The total sample size was 334 with asthma and 842 controls.

No heterogeneity was detected for OR_1 (*Gly/Gly* vs. *Arg/Arg*), OR_2 (*Arg/Gly* vs. *Arg/Arg*), or OR_3 (*Gly/Gly* vs. *Arg/Gly*) (for OR_1: $\chi^2_4 = 1.97$, $p = 0.74$; for OR_2: $\chi^2_4 = 1.38$, $p = 0.85$; for OR_3: $\chi^2_4 = 4.92$, $p = 0.30$). Logistic regression with the fixed-effect model was used to assess the overall gene effect, and this was close to the formal significance level ($LR_2 = 5.15$, $p = 0.08$). The estimated OR_1, OR_2, and OR_3 were 0.75 (95 percent CI: 0.50, 1.12), 1.08 (95 percent CI: 0.76, 1.55), and 0.70 (95 percent CI: 0.51, 0.96) (table 5). These estimates suggest a recessive protective effect of the *Gly* allele, and therefore *Arg/Arg* and *Arg/Gly* were combined and compared with *Gly/Gly*. The estimated odds ratio was 0.71 (95 percent CI: 0.53, 0.96); that is, children with the *Gly/Gly* genotype had about 29 percent lower risk of having asthma than did children with the *Arg/Arg* and *Arg/Gly* genotypes. Using the parsimonious approach gave similar results: OR_1 and OR_2 of 0.88 (95 percent CI: 0.52, 1.20) and 1.04 (95 percent CI: 0.76, 1.54), respectively. The estimated λ was −0.16 (95 percent CI: −3.85, 4.39), close to what would be expected for a recessive model, that is, 0, although the confidence interval was wide.

Gln/Glu27 polymorphism. Adult asthma. Eight studies (3, 32–36, 42, 43) assessed the association between the *Gln/Glu27* polymorphism and asthma in adult patients (table 6). The sample size was 1,162 for asthma and 1,745 for control groups. All studies except one (34) observed Hardy-Weinberg equilibrium, and seven studies were therefore pooled to assess gene effect.

Heterogeneity tests were negative for OR_1 (*Glu/Glu* vs. *Gln/Gln*) and OR_3 (*Glu/Glu* vs. *Gln/Glu*) but significant for OR_2 (*Gln/Glu* vs. *Gln/Gln*) (for OR_1: $\chi^2_6 = 2.33$, $p = 0.89$; for OR_3: $\chi^2_6 = 8.15$, $p = 0.23$; for OR_2: $\chi^2_6 = 18.47$, $p = 0.01$). A number of factors were explored, including race, but we could not identify the source of heterogeneity. We then pooled these studies by logistic regression with the random-effects model to assess the gene effect. The likelihood ratio

TABLE 4. Genotype frequencies of the *Arg/Gly16* polymorphism between asthma and control groups

Subjects, first author (reference no.)	Asthma group					Control group				
	No.	% with *Arg* allele	Genotype (no.)			No.	% with *Arg* allele	Genotype (no.)		
			Arg/Arg	*Arg/Gly*	*Gly/Gly*			*Arg/Arg*	*Arg/Gly*	*Gly/Gly*
Adults										
Arnaiz (34)*	12	54	4	5	3	39	37	9	11	19
Santillan (3)	303	45	56	163	84	604	43	101	318	185
Barr (2)	171	49	36	97	38	137	60	51	62	24
Wang (42)	128	62	52	54	22	136	51	38	64	34
Holloway (32)	154	34	29	47	78	91	40	17	39	35
Dewar (33)	117	33	14	50	53	517	40	74	263	180
Reihsaus (35)*	51	28	5	19	27	56	27	7	16	33
Hakonarson (36)	323	37	45	151	127	181	35	21	85	75
Shachor (43)	72	46	13	40	19	111	46	25	51	35
Total	1,331		254	626	451	1,872		343	909	510
Children										
Martinez (37)	38	37	5	18	15	231	35	35	108	88
Leung (41)	76	58	25	38	13	70	58	22	37	11
Binaei (38)	38	10	7	24	7	155	44	34	67	54
Lin (40)	80	58	34	35	11	69	57	27	25	17
Hopes (39)	102	37	11	54	37	317	32	28	147	142
Total	334		82	169	83	842		146	384	312

* Arnaiz and Reihsaus were not included in the pooled gene effect.

test indicated that the overall gene effect was significant (LR = 14.64, $p < 0.05$). The estimated OR_1, OR_2, and OR_3 were 0.88 (95 percent CI: 0.68, 1.14), 0.72 (95 percent CI: 0.60, 0.85), and 1.22 (95 percent CI: 0.94, 1.60) (table 5).

The estimated OR_1, OR_2, and λ by the parsimonious approach were 0.97 (95 percent CI: 0.75, 1.27), 0.88 (95 percent CI: 0.63, 1.18), and 0.61 (95 percent CI: −4.66, 5.54), respectively. Sensitivity analysis was performed by adding the one study (34) not observing Hardy-Weinberg equilibrium, and the gene effect was robust: The estimated OR_1, OR_2, and OR_3 were 0.88 (95 percent CI: 0.68, 1.13), 0.71 (95 percent CI: 0.60, 0.84), and 1.22 (95 percent CI: 0.95, 1.59), respectively This seems to indicate a homozygous or overdominant mode of effect, with heterozygotes being at lower risk of asthma than either homozygote. Pooling according to this model yielded an odds ratio of 0.73 (95 percent CI: 0.62, 0.87); that is, the chance of having asthma was about 27 percent less with *Gln/Glu* compared with *Gln/Gln* + *Glu/Glu*. Although this is a nonintuitive model, there is precedent for other genes acting in this manner (see Discussion); alternatively, this may be a spurious result due to the distribution of data and the possibility of interaction between the two polymorphic sites. We address this possibility further in the next section using haplotype analysis.

Childhood asthma. There were five studies (37–41) addressing the association between the *Gln/Glu27* polymorphism and asthma in children (table 6). All studies observed Hardy-Weinberg equilibrium except one (38).

The four studies observing Hardy-Weinberg equilibrium were pooled (37, 39–41). Since the studies by Lin et al. (40) and Leung et al. (41) had cells with no counts, we added 1 for each cell for these two studies. There was no evidence of heterogeneity for OR_1 (*Glu/Glu* vs. *Gln/Gln*), OR_2 (*Gln/Glu* vs. *Gln/Gln*), or OR_3 (*Glu/Glu* vs. *Gln/Glu*) (for OR_1: $\chi^2_3 = 0.47$, $p = 0.93$; for OR_2: $\chi^2_3 = 2.24$, $p = 0.53$; for OR_3: $\chi^2_3 = 1.51$, $p = 0.68$). Logistic regression with the fixed-effect model was then used to pool; the estimated OR_1 and OR_3 of 0.62 (95 percent CI: 0.36, 1.07) and 0.59 (95 percent CI: 0.35, 0.99), respectively, were similar, whereas the estimated OR_2 of 1.05 (95 percent CI: 0.75, 1.48) was close to one (table 5). Although the overall gene effect was not significant ($p = 0.12$), there is the suggestion of a recessive protective effect. The *Gln/Gln* and *Gln/Glu* genotypes were therefore combined and compared with *Glu/Glu*. We found that the estimated odds ratio was 0.60 (95 percent CI: 0.37, 1.00); that is, children who had the *Glu/Glu* genotype were about 40 percent less likely to have asthma than were children who had genotype *Gln/Glu* or *Gln/Gln*. Sensitivity analysis was performed by including the study not in Hardy-Weinberg equilibrium; this did not change the indication of a recessive protective effect (OR = 0.61, 95 percent CI: 0.38, 0.98). The parsimonious model was compatible with this effect, with an OR_1 of 0.90 (95 percent CI: 0.49, 1.22), an OR_2 of 1.02 (95 percent CI: 0.76, 1.40), and an estimated λ of −0.04 (95 percent CI: −3.63, 4.30). Hence, these results suggested a recessive protective effect of *Glu*, although neither model was statistically significant.

TABLE 5. Determination of the genetic effects of *Arg/Gly16* and *Gln/Glu27* polymorphisms on asthma

Genotype	Logistic regression		Model-free approach	
	Adjusted odds ratio	95% confidence interval	Adjusted odds ratio	95% confidence interval
Arg/Gly16				
Adults				
Gly/Gly vs. *Arg/Arg*	1.00	0.80, 1.24	1.01	0.79, 1.32
Arg/Gly vs. *Arg/Arg*	0.99	0.81, 1.22	1.00	0.79, 1.30
Gly/Gly vs. *Arg/Gly*	1.01	0.85, 1.20	$\lambda = 0.15$	–4.15, 4.99
Children				
Gly/Gly vs. *Arg/Arg*	0.75	0.50, 1.12	0.88	0.52, 1.20
Arg/Gly vs. *Arg/Arg*	1.08	0.76, 1.55	1.04	0.76, 1.54
Gly/Gly vs.*Arg/Gly*	0.70	0.51, 0.96	$\lambda = -0.16$	–3.85, 4.39
Gly/Gly vs. *Arg/Arg* + *Arg/Gly* (recessive effect)	0.71	0.53, 0.96		
Gln/Glu27				
Adults				
Glu/Glu vs. *Gln/Gln* (OR_1*)	0.88	0.68, 1.14	0.97	0.75, 1.27
Gln/Glu vs. *Gln/Gln* (OR_2*)	0.72	0.60, 0.85	0.88	0.63, 1.18
Glu/Glu vs. *Gln/Glu* (OR_3*)	1.22	0.94, 1.60	$\lambda = 0.61$	–4.66, 5.54
Gln/Glu vs. *Gln/Gln* + *Glu/Glu* (overdominant effect)	0.73	0.62, 0.87		
Glu/Glu vs. *Gln/Gln* (OR_1)	0.62	0.36, 1.07	0.90	0.49, 1.22
Gln/Glu vs. *Gln/Gln* (OR_2)	1.05	0.75, 1.48	1.02	0.76, 1.40
Glu/Glu vs. *Gln/Glu* (OR_3)	0.59	0.35, 0.99	$\lambda = -0.04$	–3.63, 4.30
Glu/Glu vs. *Gln/Glu* + *Gln/Gln* (recessive effect)	0.60	0.37, 1.00		

* OR_1, odds ratio of asthma with the preceding comparison of genotypes (OR_2 and OR_3 defined similarly).

Haplotype analysis of *Arg/Gly16* and *Gln/Glu27* polymorphisms

Three studies of adults provided data for haplotype analysis (3, 33, 36). The study by Weir et al. (30) reported inferred haplotype data among subjects who had only homozygous wild or mutant genotypes at one locus, so this study was not included in the present analysis. The expectation-maximization algorithm was applied to infer haplotypes for the three studies, and linkage disequilibrium was assessed. The estimated linkage disequilibrium coefficient was 0.48 ($p < 0.001$).

The haplotype frequency in asthmatics and controls is described in table 7. The three most common haplotypes were *Arg/Gln* (37.5 percent), *Gly/Glu* (31.7 percent), and *Gly/Gln* (28.2 percent). The estimated odds ratios were 0.39 (95 percent CI: 0.29, 0.58), 0.99 (95 percent CI: 0.74, 1.49), and 0.83 (95 percent CI: 0.62, 1. 24) for haplotypes *Arg/Glu*, *Gly/Gln*, and *Gly/Glu* compared with *Arg/Gln*. These numbers seem to indicate that, when *Gln* is present at position 27, the risk of asthma is the same regardless of what allele is present at position 16. However, with *Glu* at position 27, the risk of asthma is lower, and this decreased risk is modified by the allele at position 16, being lower with *Arg16* than with *Gly16*.

This effect modification is marked, and the confidence interval of the odds ratio for the *Arg/Glu* haplotype does not overlap with that of the *Gly/Glu* haplotype (table 7). Subjects who had haplotypes *Arg/Glu* and *Gly/Glu* were 61 percent and 17 percent less likely to have asthma than were subjects who had haplotype *Arg/Gln*. However, subjects with haplotype *Gly/Gln* had the same chance of asthma as did subjects with *Arg/Gln*.

DISCUSSION

The various results of the individual single-nucleotide polymorphism (SNP) analyses and haplotype analyses are complex, but synthesizing the data overall seems to indicate the following. First, the *Glu27* allele appears to be protective against asthma, reducing the risk of asthma by approximately 27 percent. This makes biologic sense because the *Glu* variant is resistant to down regulation in vitro, and it is possible that these individuals express higher β_2-receptor levels in the context of inflammation. This was suggested

TABLE 6. Genotype frequencies of the *Gln/Glu27* polymorphism between asthma and control groups

Subjects, first author (reference no.)	Asthma group					Control group				
	No.	% with *Gln* allele	Genotype (no.)			No.	% with *Gln* allele	Genotype (no.)		
			Gln/Gln	*Gln/Glu*	*Glu/Glu*			*Gln/Gln*	*Gln/Glu*	*Glu/Glu*
Adults										
Arnaiz (34)*	12	58	6	2	4	39	51	14	12	13
Santillan (3)	303	88	241	53	9	604	80	385	202	17
Wang (42)	128	92	108	19	1	136	91	113	22	1
Holloway (32)	153	87	49	76	28	91	59	35	37	19
Dewar (33)	119	49	33	51	35	511	53	134	271	106
Reihsaus (35)	51	39	13	26	12	56	51	17	23	16
Hakonarson (36)	324	55	92	173	59	199	52	48	112	39
Shachor (43)	72	73	38	29	5	109	69	50	50	9
Total	1,162		580	429	153	1,745		796	729	220
Children										
Martinez (37)	38	64	16	17	5	231	64	95	104	32
Hopes (39)	102	54	24	63	15	317	51	83	156	78
Leung (41)	76	92	64	12	0	70	89	55	15	0
Binaei (38)*	37	78	23	12	2	155	81	107	36	12
Lin (40)	80	91	65	15	0	69	88	54	14	1
Total	333		192	119	22	842		394	325	123

* Not in Hardy-Weinberg equilibrium and not pooled.

in both adult and pediatric populations, although the genetic model in each was different.

Second, the protective effect of *Glu27* may be due to the haplotype. It is probable that this is not an effect of this SNP in isolation but, instead, reflects a common haplotype that includes this allele. Drysdale et al. (44) investigated 13 SNPs in the human β_2-adrenergic receptor gene promoter and coding regions in relation to responsiveness to β_2 agonists. They found that, although there was no association when SNPs were analyzed individually, there was a clear relation between one of the common haplotypes (haplotype 2 in their paper, which included *Glu27*) and good response to β_2 agonists in vivo, as well as increased messenger RNA levels and gene expression in vitro. Haplotypes that included *Gln27* (e.g., haplotype 4 in their paper) had overall poorer response to β_2 agonists and lower expression levels. Presumably, good response to exogenous agonists also reflects good response to endogenous agonists and, hence, a protective effect against asthma.

Third, the genetic model suggested by the data appears to be an overdominant protective effect of *Glu27*. This model is also called heterozygote advantage or positive heterosis, and although it may appear counterintuitive, a recent review indicates that this mode of action is perhaps more common than previously thought and cites numerous examples (45). Indeed, the *IL12B* promoter polymorphism has been associated with severity of asthma in children, and this also seems to observe a pattern of heterozygote advantage (46). The mechanism of such a model is still speculative but may include 1) advantages in having variation in a multimeric protein, such as better V_{max} (47); 2) an allele with a selective advantage that is detrimental when homozygous (e.g., sickle

TABLE 7. Distribution of haplotype frequency of *Arg/Gly16* and *Gln/Glu27* polymorphisms between asthma and control groups

Haplotype	Control group ($n = 2,331$)		Asthma group ($n = 950$)		Adjusted odds ratio*	95% confidence interval
	No.	%	No.	%		
Arg/Gln	978	37	573	39	1.00	
Arg/Glu	91	3	18	1	0.39	0.29, 0.58
Gly/Gln	741	28	428	29	0.99	0.74, 1.49
Gly/Glu	852	32	461	31	0.83	0.62, 1.24

* Adjusted for study effect.

cell and falciparum malaria); and 3) a greater range of expression of gene products and plasticity with heterozygotes than homozygotes (45). Alternatively, this may be a spurious result due to other untyped loci in the haplotypes analyzed.

Fourth, there may be interaction or synergism between different SNPs. The haplotype analysis raises the possibility that the position 16 polymorphism may be an effect modifier: The protective effect of *Glu27* was accentuated with *Arg16* compared with *Gly16,* although there was no independent effect of the position 16 polymorphism on its own. This would indicate that it may be difficult to predict a haplotype effect from its constituent SNPs.

Fifth, the linkage disequilibrium between position 16 and 27 polymorphisms is not high. This may be surprising given that they are only 30 nucleotides apart and there are no intervening introns. However, this is congruent with other studies indicating that recombination frequency is not strictly proportional to chromosomal distance, and it is sensitive to ancestral effects; for example, Drysdale et al. found that ''some pairs of close sites have reduced levels of linkage disequilibrium relative to more spaced pairs of sites'' (44, p. 10485).

The pooled allele frequencies at both the *Arg16* and *Gln27* sites confirm the presence of significant variation between racial groups and are similar to values generally recognized, for example, in ALFRED (Allele Frequency Database) (48). Although crude, these results do support a role of these polymorphisms in asthma susceptibility, given the varying incidence of asthma in these racial groups. Interestingly, the variation was more marked at the *Gln27* locus than at *Arg16*, and it was the former that was more strongly implicated in asthma susceptibility in our results.

These findings must be taken with caution at the present time for a number of reasons. First, these estimates are obtained by pooling despite heterogeneity.

Second, the asthma phenotype was often not fully specified, and details of asthma diagnoses were often scanty. Future studies should clearly identify whether asthma cases were diagnosed from symptoms or on population screening, and they should include results of atopic testing, spirometry, or methacholine challenge. Without sufficient information in individual studies, the condition labeled as asthma in this meta-analysis is likely to be heterogeneous and may be contributing to the inconsistency of results.

Third, the haplotype results are very different from those found in the longitudinal Normative Aging Study cohort (49), where the *Gly16/Gln27* haplotype had a protective effect compared with *Arg16/Glu27* (a different reference genotype), whereas in our study there was an increased risk. This discrepancy, however, may be due to the fact that, in the latter, the outcome was airway hyperresponsiveness (which does not always correspond to asthma) and that the population was general, community-dwelling males screened with a methacholine challenge test, not diagnosed asthmatics.

Fourth, these findings do not take into account smoking status, since data were available from only two studies (3, 42). There are some indications that the genotype effects may be more apparent among nonsmokers (49).

Fifth, the findings in childhood and adult asthma are inconsistent. This may be due to chance, or, alternatively, there may be a genuinely different mode of action in adults compared with children, in that asthma is a clinically different disease in these two populations. Asthma in late childhood, which was the age range studied in these papers, is predominantly atopic in nature, more likely to be eosinophilic, more likely to be symptom diagnosed and episodic, and less likely to be associated with persistent airway hyperresponsiveness (49–52). Since the *Glu27* polymorphism is associated with less airway hyperresponsiveness (53), this may explain differences between the associations in adults and children. Alternatively, given the incomplete understanding of asthma pathogenesis, there may be pleiotropic effects of the β_2-receptor at different stages or etiologies of disease. Indeed, one of us has observed such an age-specific association for another gene candidate in a population of children followed from childhood into early adult life (54).

In summary, these results are suggestive of a protective effect of the *Glu27* allele, probably as part of a haplotype, and they raise the possibility of interactions with the position 16 alleles and possibly other SNPs. This warrants further investigation in larger studies. The clinical implications of these findings are not clear. These polymorphisms may be involved in both conferring the risk to develop asthma and influencing the response to β_2-agonists; this has been the subject of a recent randomized crossover trial (55) and is the topic of an ongoing meta-analysis (56).

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APPENDIX

The pooled prevalence was calculated as

$$\bar{p} = \frac{\sum w_i p_i}{\sum w_i},$$

where $\bar{p}$ was the pooled prevalence of the allele, p_i was the prevalence of the allele in each study, and w_i was $1/\text{var}(p_i)$, which was the weight of each study.

Heterogeneity of prevalences across studies was checked as follows:

$$Q = \sum w_i (p_i - \bar{p})^2.$$

The Q statistic follows a χ^2 distribution with number of studies (k) – 1 df. If heterogeneity was present, between-study variation was then estimated as follows:

$$\tau^2 = \frac{Q - (k-1)}{\sum w_i - \frac{\sum w_i^2}{\sum w_i}} \quad \text{if } Q > k-1 \text{ or 0 otherwise.}$$

This was used to calculate a weight term that accounted for between-study variation:

$$w_i^* = \frac{1}{\text{var}(p_i) + \tau^2},$$

and the pooled prevalence was estimated as follows:

$$\overline{p^*} = \frac{\sum w_i^* p_i}{\sum w_i^*}.$$

The 95 percent confidence interval was estimated as follows:

$$95 \text{ percent CI} = \overline{p^*} \pm \frac{1.96}{\sqrt{\sum w_i^*}}.$$

APPENDIX TABLE 1. Scale for quality assessment of molecular association studies of asthma

Criteria	Score
Representativeness of cases	
Consecutive/randomly selected from case population with clearly defined sampling frame	2
Consecutive/randomly selected from case population without clearly defined sampling frame or with extensive inclusion/exclusion criteria	1
No method of selection described	0
Representativeness of controls	
Controls were consecutive/randomly drawn from the same sampling frame (ward/community) as cases	2
Controls were consecutive/randomly drawn from a different sampling frame as cases	1
Not described	0
Ascertainment of asthma	
Clearly described objective criteria for diagnosis of asthma	2
Diagnosis of asthma by patient self-report or by patient history	1
Not described	0
Ascertainment of controls	
Controls were tested to screen out asthma, i.e., measured FEV_1* or PEFR*	2
Controls were subjects who did not report asthma; no objective testing	1
Not described	0
Genotyping examination	
Genotyping done under "blinded" condition	1
Unblinded or not mentioned	0
Hardy-Weinberg equilibrium	
Hardy-Weinberg equilibrium in control group	2
Hardy-Weinberg disequilibrium in control group	1
No checking for Hardy-Weinberg equilibrium	0
Association assessment	
Assess association between genotypes and asthma with appropriate statistics and adjustment for confounders	2
Assess association between genotypes and asthma with appropriate statistics without adjustment for confounders	1
Inappropriate statistics used	0
Response rate	
Response rates for both groups are the same, i.e., to within 5%	2
Response rates are different, between 5% and 10%	1
Response rates are more than 10% different, or no mention of response rates	0
Total	

* FEV_1, forced expiratory volume in 1 second; PEFR, peak expiratory flow rate.